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Extended Abstract

Nanomedicine & Drug Delivery : Electron microscopic visualization and recording of myosin head recovery and power strokes in hydrated myosin filaments using the gas environmental chamber- Haruo Sugi - Teikyo University, Japan

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Abstract

Although more than 50 years have passed since the monumental discovery of sliding filament mechanism in muscle contraction, the molecular mechanism of myosin head movement, coupled with ATP hydrolysis, is still a matter for debate and speculation. Most straightforward way to study myosin head movement, producing myofilament sliding, may be to directly record ATP-induced myosin head movement in hydrated, living myosin filaments using the gas Environmental Chamber (EC) attached to an electron microscope. While the EC has long been used by materials scientists for the in situ observation of chemical reaction of inorganic compounds, we are the only group successfully using the EC to record myosin head movement in living myosin filaments. We position-mark individual myosin heads by attaching gold particles (diameter, 20 nm) via three different monoclonal antibodies, attaching to: At the distal region of myosin Head Catalytic Domain (CAD); at the myosin head Converter Domain (COD) and at the myosin head Lever arm Domain (LD). First, we recoded ATP-induced myosin head movement in the absence of actin filaments and found that myosin heads moved away from the central bare region of myosin filaments. This finding constitutes the first direct electron microscopic recording of myosin head recovery stroke under a condition in which myosin heads almost freely with average amplitude of ~7 nm. After many efforts, we succeeded in recording ATP-induced myosin head power stroke in actin-myosin filament mixture in 2015. Since only a limited proportion of myosin heads can be activated by a limited amount of ATP applied, myosin heads only move by stretching adjacent sarcomere structures, i.e., nominally isometric condition. Myosin head CAD did not move parallel to the filament axis in the standard ionic strength, while it moved parallel to the filament axis at low ionic strength, in accordance with our physiological experiments on single muscle fibers. These results indicate that myosin head movement does not necessarily obey predictions of the swinging lever arm hypothesis appearing in every textbook

In 1954, H.E. Huxley and Hanson made a monumental discovery that muscle contraction is produced by the sliding between actin and myosin filaments, which is believed to be caused by cyclic attachment and detachment between myosin heads extending from myosin filaments and the corresponding myosin-binding sites in actin filaments. Muscle is regarded as a machine to convert chemical energy of ATP hydrolysis into mechanical work

The myosin head (M) in the form of M–ADP–Pi first binds with an actin filament (A) to form A–M–ADP–Pi (A), and then changes its configuration to perform the power stroke, associated with the reaction, A–M–ADP–Pi → A–M + ADP + Pi, to produce unitary sliding between the actin and myosin filaments (A to B). After completion of the power stroke, M remains bound to A until the next ATP comes to bind to it (B). On binding with ATP, M detaches from A (C), and performs a recovery stroke associated with the reaction, M–ATP → M–ADP–Pi, to return to its original configuration (C to D). In order to repeat cyclic changes in configuration, the power and recovery strokes should be the same in amplitude, and opposite in direction. Despite extensive studies on contracting muscle using methods of muscle mechanics, chemical probes attached to myosin heads, and time-resolved X-ray diffraction, it has not been possible to clearly prove and characterize the myosin head power and recovery strokes mainly because of the asynchronous nature of myosin head movement

The most straightforward means to study the power and recovery strokes in individual myosin heads is to record their movement in response to ATP electron microscopically. It has been a dream of life scientists to observe living organisms under an electron microscope, and a number of attempts have been made to study living microorganisms in aqueous environment electron-microscopically. Such attempts were, however unsuccessful because of electron beam damage that destroyed cell functions. Meanwhile, it seemed possible to study dynamic structural changes of biomolecules, such as actin and myosin, using a gas environmental chamber (EC), in which biological specimens are insulated from the high vacuum of electron microscopes and are kept in aqueous solution. Fortunately, we have been able to use an EC system,

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developed by the late Professor Akira Fukami of Nihon University with the aid of Japan Electron Optics Laboratory, Ltd. (JEOL, Akishima, Tokyo, Japan), and we succeeded in visualizing and recording ATP-induced myosin head movements in hydrated, living myosin filaments electron microscopically. In this article, we only describe the results obtained from actin and myosin filaments of rabbit psoas muscle fibers, though we have also made preliminary experiments using myosin–paramyosin core complex filaments White male rabbits (2–2.5 kg) were killed by injection of sodium pentobarbital (50 mg/kg) into the ear vain, and psoas muscles were dissected from the animals. Actin (F-actin) and myosin were prepared from the psoas muscle. The specimens used for the experiments described in this section were synthetic myosin filaments consisting of a myosin–myosin rod mixture, prepared by mixing myosin and myosin rod at a molar ratio of 1:1 and then slowly polymerizing them by dialysis against a low ionic strength solution. Further details of the methods have been described elsewhere

The EC experiments provide only means to directly visualize and record ATP-induced movement of individual myosin heads, since all other method can only study myosin head movement in concert based on various assumptions, which are not necessarily proved to be valid. On the basis of the results obtained from the EC experiments and the freeze-fracture studies on the myosin head configurations before and during the power and recovery strokes, myosin head movements can be summarized as follows. In relaxed muscle, individual myosin heads fluctuate over a large distance around a definite neutral configuration; In the neutral configuration, the myosin head CAD is perpendicular to the axis of actin and myosin filaments; In the absence of actin filaments, individual myosin heads can perform a recovery stroke in the direction away from myosin filament central bare region (recovery stroke), without being guided by actin filaments; The mean amplitude of the recovery stroke, in the absence of actin filaments, is the same (~6 nm) at both the distal and the proximal regions of the myosin head CAD; In the actin–myosin filament mixture, in which only a small fraction of myosin heads can be activated by a limited amount of ATP applied, individual myosin heads perform a power stroke by stretching adjacent elastic structures; The mean amplitude of the power stroke is 3.3 nm at the distal region and 2.5 nm at the proximal region of the myosin head CAD at the standard ionic strength, so that the myosin head CAD is oblique to both the actin and myosin filaments; At low ionic strength, which is known to enhance Ca2+-activated isometric tension ~twofold, the mean amplitude of the power stroke increases to 5 nm at both the distal and the proximal regions of the myosin head CAD, so that the myosin head CAD is perpendicular to both the actin and myosin filaments during the course of the stroke.

To summarize, when myosin heads hydrolyse ATP to form M–ADP–Pi (charged-up state), they perform a recovery stroke in the absence of actin filaments, and a power stroke in the presence of actin filaments; in both cases, they return to their neutral position after release of Pi and ADP. The EC experiments described above are extremely promising for future studies of the remaining mysteries in muscle contraction. For example, if the EC methods are coupled with those of laser flash photolysis of caged ATP and time-resolved electron microscopy , we will be able to make a remarkable progress towards the full understanding of muscle contraction mechanisms. We are now planning to perform experiments with these methods.

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